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13. ABSTRACT *(Maximum 200 Words)*

The purpose of this Sabbatical Training Grant from the USAMRMC Breast Cancer Program was to provide the training, dedicated time, and computational resources necessary for the development of a research program on Drug Discovery and Structural Bioinformatics in Breast Cancer. The development of innovative therapeutic and/or chemopreventive approaches for breast cancer will involve the identification of novel macromolecular targets, the discovery of novel lead drug candidates, and drug design and development. The biological focus of our research addresses estrogen biosynthesis and on estrogen-induced gene expression in hormone-dependent breast cancer. Identification of critical small molecule-protein and protein-protein interactions during gene expression and signal transduction in the areas of steroidogenesis and estrogen-induced responses will result in new molecular targets for drug discovery and design for the treatment of hormone-dependent breast cancer. The Sabbatical Training Grant provided an enhancement of our research endeavors by utilizing computational and experimental tools for macromolecular target identification, target validation, and lead identification. The objectives of the funded grant were accomplished: (1) obtained the computational resources (hardware, software) for database searching, computational analysis, structure prediction, and homology-based structure modeling; (2) developed expertise in peptide and protein model building, homology-based protein construction, 3-D conformational analysis, and receptor pharmacophore modeling through intensive research-based workshops, on-site training, and extended visits to national laboratories; (3) applied these structural bioinformatics approaches to the analysis of gene and protein sequences involved in macromolecular interactions in breast cancer; and (4) initiated medicinal chemistry and biochemistry research using subcellular and cell culture studies for target validation and discovery of novel lead candidates.

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FOREWORD

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N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Robert W Bruegge 7/17/00

PI - Signature Date

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INTRODUCTION

The purpose of this Sabbatical Training Grant from the USAMRMC Breast Cancer Program was to provide the training, dedicated time, and computational resources necessary for the development of a research program on Drug Discovery and Structural Bioinformatics in Breast Cancer. The development of innovative therapeutic and/or chemopreventive approaches for breast cancer will involve the identification of novel macromolecular targets, the discovery of novel lead drug candidates, and drug design and development. The "biological" focus of our research addresses estrogen biosynthesis and on estrogen-induced gene expression in hormone-dependent breast cancer. Identification of critical small molecule-protein and protein-protein interactions during gene expression and signal transduction in the areas of steroidogenesis and estrogen-induced responses will result in new molecular targets for drug discovery and design for the treatment of hormone-dependent breast cancer. The Sabbatical Training Grant provided an enhancement of our research endeavors by utilizing computational and experimental tools for macromolecular target identification, target validation, and lead identification. The objectives of the funded grant were accomplished:

1. Obtained the computational resources (hardware, software) for database searching, computational analysis, structure prediction, and homology-based structure modeling.

2. Developed expertise in peptide and protein model building, homology-based protein construction, 3-D conformational analysis, and receptor pharmacophore modeling through intensive research-based workshops, on-site training, and extended visits to national laboratories.

3. Applied these structural bioinformatics approaches to the analysis of gene and protein sequences involved in macromolecular interactions in breast cancer.

4. Initiated medicinal chemistry and biochemistry research using subcellular and cell culture studies for target validation and discovery of novel lead candidates.

BODY OF TRAINING REPORT

Cancer is the leading cause of death among women between the ages of 30 and 54. An estimated 186,000 new cases of breast cancer will be diagnosed, and 46,000 women in the U.S. will die from breast cancer in 1997. Currently, one out of nine American women will develop breast cancer in her lifetime. The rate of breast cancer incidence has increased 2-3% per year over the past decade in both premenopausal and postmenopausal women. This research focuses on examination of the role of steroid hormones in breast cancer etiology. An estimated 60-70% of human breast cancers are associated with sex hormone exposure. Approximately 60% of all breast cancer patients have hormone-dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth. The possible biochemical roles of estrogens in the development of breast cancers remains to be fully elucidated. Estradiol (E_2) is the most potent endogenous estrogen and is biosynthesized from androgens by the cytochrome P450 enzyme complex called aromatase. Estradiol produces normal physiological effects by binding to a specific nuclear receptor protein, the estrogen receptor (ER). Estrogens promote growth of estrogen responsive (ER+) breast cancer cells, and production of growth factors by estrogens in established tumor cell culture lines has been demonstrated in the past few years. Recent research on regulation of steroidogenic enzyme expression such as aromatase and on regulation of ER-mediated responses suggest critical roles for protein-protein interactions during gene expression. As these biochemical mechanisms become more fully understood, new macromolecules and biochemical pathways involving protein-protein interactions will be identified and serve as new molecular targets for drug discovery and design.

The development of innovative therapeutic and/or chemopreventive approaches for breast cancer involves the identification of novel macromolecular targets, the discovery of novel lead drug candidates, and drug design and development. The purpose of this Sabbatical Training Grant has been to provide the training, dedicated time, and computational resources necessary for the development of a research program on **Drug Discovery and Structural Bioinformatics in Breast Cancer**. This grant provides an enhancement of our research endeavors by utilizing computational and experimental tools for macromolecular target identification, target validation, and lead identification.

The accomplishments of this Sabbatical Training Grant from the USAMRMC Breast Cancer Program are grouped here according to the objectives of the original grant application:

Objective 1: **Obtain the computational resources (hardware, software) for database searching, computational analysis, structure prediction, and homology-based structure modeling.**

The computer workstation, Silicon Graphics O_2, was purchased and installed in September 1998. The SGI O_2 workstation was equipped with 200 MHz R5000, 1MB SC, 64MB memory, 4GB hard disk, 17" monitor, video I/O, and an O_2 digital camera. Hardware upgrades, including the addition of a second hard drive (9GB), ZIP and JAZ drives, and a laser printer, were completed by February 1999. The molecular modeling software, Tripos SYBYL Version 6.5, was installed in November 1998, a license upgrade was purchased and installed in November 1998, and the Composer Module was added in January 1999. The software was updated with SYBYL Version 6.6.1, installed in May 2000.

Objective 2: **Develop expertise in peptide and protein model building, homology-based protein construction, 3-D conformational analysis, and receptor pharmacophore modeling through intensive research-based workshops, on-site training, and extended visits to national laboratories.**

I attended intensive training workshops entitled "Biopolymer Modeling" and "Structure-Based Drug Design" at the Tripos facility in St. Louis, MO, on October 26 and 27, 1998. At that time, these workshops are offered once per year and were held in early 1998 (which I was not able to attend). I was able to arrange a unique opportunity to go to the home facility in St. Louis, and I worked one-on-one with a Tripos instructor, Dr. Thomas P. Jones. The two workshops involved a series of computational chemistry and molecular modeling exercises designed to explore and understand the capabilities and strengths, as well as the limitations, of the SYBYL and its Biopolymer, Composer, and UNITY modules. Additionally, TRIPOS was in the process of re-doing their workshops and we ran through a number of new exercises they were introducing for the 1999 workshops. The "Biopolymer Modeling" workshop involved force field comparisons, molecular dynamics, building protein loops, sequence alignment methods, and homology modeling. The "Structure-Based Drug Design" workshop involved preparation of receptor target, flexible docking of ligands, database searching from a receptor site, conformational analysis, and receptor-based *de novo* design.

In addition, I attended four meetings specifically emphasizing drug discovery and bioinformatics and attended one national American Chemical Society meeting:

(a) **3rd Lake Tahoe Symposium on Molecular Diversity**, Lake Tahoe, CA, from January 24 to 29, 1999. I served as chair of one session at the meeting, and one of my graduate students presented our research on benzopyrones at the meeting.

(b) **Discover '99**, San Diego, CA, from April 26 to 29, 1999.

(c) **Exploring Molecular Diversity** and **High Throughput Organic Synthesis** conferences, Hyatt Regency, La Jolla, CA, February 9 - 11, 2000. I presented a symposium lecture entitled "Synthetic Approaches to Benzopyrone Combinatorial Libraries for Identification of Selective Bioactive." I also served as chair of one session at the meeting.

(d) **4th Lake Tahoe Symposium on Molecular Diversity**, Lake Tahoe, CA, from March 19 to 24, 2000. I served as chair of one session at the meeting.

(e) **218th American Chemical Society Meeting**, New Orleans, LA, August 22-26, 1999. One of my graduate students presented our research on benzopyrones at the meeting.

I also visited the National Cancer Institute (NCI) and the National Center for Biotechnology Information (NCBI) to discuss cancer drug discovery and bioinformatics from March 1 to 3, 1999. While in Washington, DC, I participated in a half-day symposium on sabbatical leaves at the annual AACP meeting on March 1, 1999.

Objective 3: **Apply these structural bioinformatics approaches to the analysis of gene and protein sequences involved in macromolecular interactions in breast cancer.**

I began using SYBYL Composer to model proteins critical for growth of hormone-dependent breast cancer, i.e., aromatase and the estrogen receptors. Initial efforts have focused on development of molecular models for aromatase and for estrogen receptor α (ERα). Studies include the identification of critical amino acids at the active site, examination of interactions with both steroidal and nonsteroidal inhibitors, and design of new analogs.

The ERα ligand binding domain (LBD) crystal structures were obtained from the Brookhaven Protein Database (1ERE; 3ERD; 1ERR; 3ERT) and used in the modeling. Active site residues were identified and defined in the FlexiDock software to be within 4Å of site for ligand binding. SYBYL 6.6.1, Biopolymer, SYBYLDock, and FlexiDock were used to investigate the binding of steroids (estradiol) and nonsteroidal agents (diethylstilbestrol, 4-hydroxytamoxifen, raloxifene, and several benzopyranone molecules). The ligand binding domain was prepared using Biopolymer module. Ligands were constructed and optimized using SYBYL sketch function. Docking of ligands to estrogen receptor's binding site was performed with Biopolymer and FlexiDock module. The prospective hydrogen bonding sites in ligands and ligand binding site were set prior to start docking step. Using this molecular modeling approach, we determined that (a) nonsteroidal benzopyranones are able to bind effectively into the binding site for a ligand and (b) the relative strength of binding at the site is dependent on the ability to form hydrogen bonds with critical amino acid residues. For example, the FlexiDock relative binding energy for estradiol in these modeling calculations is approximately -772.90 kcal/mol. Hydrogen bonds to the 3-phenolic group and the 17β-hydroxyl group provide for enhanced binding and thus a low binding energy. Genistein, a nonsteroidal isoflavonoid benzopyranone, produces a FlexiDock relative binding energy of -328.51 kcal/mol. The calculations for a simple benzopyranone molecule (without critical hydroxyl groups) provide a FlexiDock relative binding energy of -288.91 kcal/mol. Graphical representations of these molecules bound to the ligand binding site are provided in the Appendix.

The molecular models for aromatase are more complicated in that a crystal structure for mammalian cytochrome P450 proteins is not available due to the problems associated with crystallizing membrane proteins. Only crystal structures for soluble cytochrome P450 proteins from certain bacterial enzymes are available, and these structures are used in homology modeling. Although the overall shapes of the proteins are consistent, the detail structures of the substrate binding sites are significantly different with each cytochrome P450 protein. An aromatase model was constructed by homology modeling with crystal structure coordinates of cytochrome P450-BM3 and cytochrome P450-terp using Biopolymer module. A second molecular model for aromatase was also determined using SWISS-MODEL available from the Swiss Institute for Bioinformatics through the ExPASy proteomics server available on the web site (http://www.expasy.ch). Active site residues were identified and defined in the FlexiDock software to be within5Å of heme iron. Using this molecular modeling approach, we determined that certain nonsteroidal benzopyranones are able to bind effectively into the binding site for a

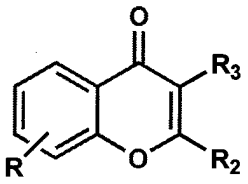
ligand, and graphical representation of a simple benzopyranone molecule (without critical hydroxyl groups) bound to the substrate binding site is provided in the Appendix.

Also, preliminary studies were initiated on the three-dimensional structures of growth factor proteins (such as KGF and FGF-10) in order to design small molecule receptor antagonists. Finally, molecular spreadsheets (MMS) were developed for benzopyranones as potential antagonists for aromatase and estrogen receptors.

Objective 4: **Initiate medicinal chemistry and biochemistry research using subcellular and cell culture studies for target validation and discovery of novel lead candidates.**

Our molecular modeling studies indicate that the low molecular weight, fairly rigid benzopyranone nucleus is easily accommodated in the binding sites of ERα and aromatase. Molecules with the benzopyranone ring system include a number of natural products such as flavonoids found widely in higher plants. Considerable interest in flavonoids in breast cancer has been stimulated by the hypothesis that these natural products are dietary factors that may be responsible for the lower incidence of breast cancer in women from certain regions of the world. The chemical class of flavonoids encompasses the flavones, isoflavones, flavanones, and flavonols. Compounds in this class have shown activity as protein tyrosine kinase inhibitors, estrogen receptor agonists/antagonists, or inhibitors of estrogen biosynthesis. This basic benzopyranone chemical structure contains multiple sites of potential diversity and serves as an ideal template for combinatorial libraries.

Our hypothesis is that the design, synthesis, and screening of substituted 4*H*-benzopyran-4-one combinatorial libraries will allow us to harvest the biological potential of these molecules and develop more selective agents for molecular targets (estrogen receptors; aromatase) in breast cancer. The libraries will contain three types of chemical diversity on the molecules (Figure 1.).



R = Alkyl, OH, OR, NHR, Halogens
R_3, R_2 = aromatic, heteroaromatic, alkyl, cycloalkyl

Figure 1.

Bioassay determinations have been initiated on the initial benzopyranones molecules, including examination of effects on breast cancer cell proliferation, estrogen receptor affinities, and aromatase inhibition. Compounds that stimulate and that inhibit the growth of estrogen-responsive MCF-7 cells (Figure 2) have been identified as possible lead candidates,

and additional synthetic medicinal chemistry is now underway to enhance the biological activities and lead to more potent and selective agents.

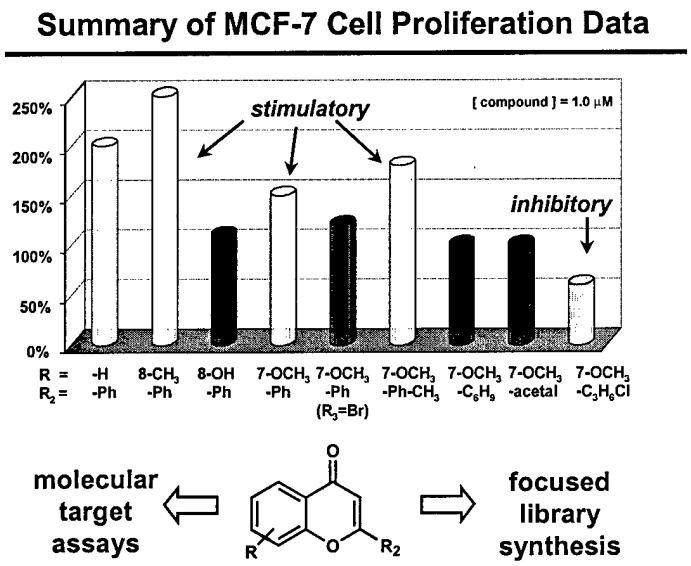


Figure 2.

KEY RESEARCH ACCOMPLISHMENTS:

- Obtained SGI O_2 workstation and molecular modeling software for database searching, computational analysis, structure prediction, and homology-based structure modeling.
- Developed expertise in protein model building, homology-based protein construction, 3-D conformational analysis, and receptor pharmacophore modeling through intensive research-based workshops, on-site training, and visits to national laboratories.
- Applied these structural bioinformatics approaches to the analysis of the macromolecular interactions of steroidal and nonsteroidal molecules with ERα ligand binding domain.
- Developed a molecular model of aromatase using homology-based structure modeling and evaluated the macromolecular interactions with nonsteroidal benzopyranones.
- Using this molecular modeling approach, we determined that (a) nonsteroidal benzopyranones are able to bind effectively into the binding site for a ligand and (b) the relative strength of binding at the site is dependent on the ability to form hydrogen bonds with critical amino acid residues.
- The design, synthesis, and screening of 4*H*-benzopyran-4-one combinatorial libraries have been initiated in order to harvest the biological potential of these molecules and develop more selective agents for estrogen receptors and for aromatase in breast cancer. This research is now funded by USAMRMC grant DMAD17-00-1-0388.

APPENDIX

A. Molecular Modeling Figures

Figure 1. E_2 in binding site of ERα LBD

Figure 2. Benzopyrone in binding site of ERα LBD, orientation A

Figure 3. Benzopyrone in binding site of ERα LBD, orientation B

Figure 4. Benzopyrone in binding site of Aromatase

C. Manuscripts: The following manuscripts were published or are currently in press from research supported in part by this Sabbatical Training Grant.

1. A.S. Bhat, J.L. Whetstone, and **R.W. Brueggemeier**, Novel synthetic routes suitable for constructing benzopyrone combinatorial libraries. *Tetrahedron Letters*, **40**, 2469-2472 (1999).
2. A. S. Bhat, J. L. Whetstone, and **R. W. Brueggemeier**, A method for the rapid synthesis of benzopyrone libraries employing a resin capture strategy. *J. Combi. Chem.*, accepted (2000).

D. Presentations: The following presentations related to the Sabbatical Training Grant were given during this period. The presenter is indicated with an underlined name.

1. A.S. Bhat, J.L. Windholtz, and **R.W. Brueggemeier**, Novel approaches for the synthesis of diverse benzopyrone libraries. 3rd Lake Tahoe Symposium on Molecular Diversity, Lake Tahoe, CA, Jan. 24-29, 1999.
2. **R.W. Brueggemeier**, Perspectives on Sabbatical Leave, AACP Interim Meeting, Washington, DC, March 1, 1999.
3. J.L. Whetstone, A.S. Bhat, and **R.W. Brueggemeier**, Benzopyrone as a scaffold for combinatorial libraries. 1999 National Organic Symposium, Madison, WI, June 13-17, 1999, Abst. #287.
4. J.L. Whetstone, A.S. Bhat, and **R.W. Brueggemeier**, Synthetic routes for diversifying benzopyrones in combinatorial libraries. 218th American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999, Abst. MEDI #140.
5. **R.W. Brueggemeier**, J.L. Whetsone, A.S. Bhat, and S. Joomprabutra, Benzopyrone combinatorial libraries for identification of selective agents for molecular targets in breast cancer. Molecular Targets in Cancer Therapeutics, AACR-NCI-EORTC International Conference, Washington, DC, November 16-19, 1999, Abst. #321.
6. **R.W. Brueggemeier**, Synthetic approaches to benzopyrone combinatorial libraries. Exploiting Molecular Diversity/High-Throughput Organic Synthesis, Cambridge Healthtech Institute, San Diego, CA, Feb. 10, 2000.

7. **R.W. Brueggemeier**, Molecular modeling, steroid biochemistry, and drug design. 2000 DoD Era of Hope Breast Cancer Research Program Meeting, Atlanta, GA, June 8-12, 2000, Poster CC-26.

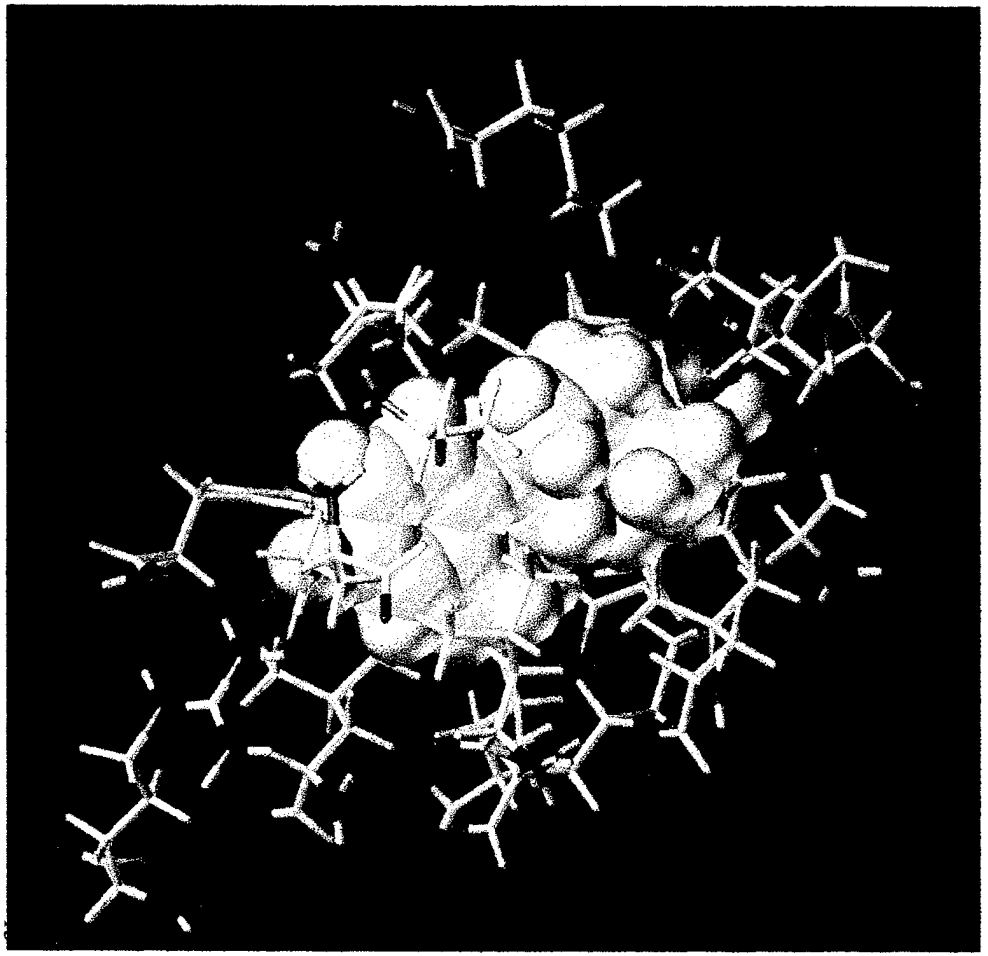
8. **R.W. Brueggemeier**, J.L. Whetstone, A.S. Bhat, and S. Joomprabutra, Combinatorial libraries for identification of nonsteriodal agents for breast cancer targets. 14th International Symposium of the *Journal of Steroid Biochemistry and Molecular Biology*, Quebec City, Canada, June 24-27, 2000.

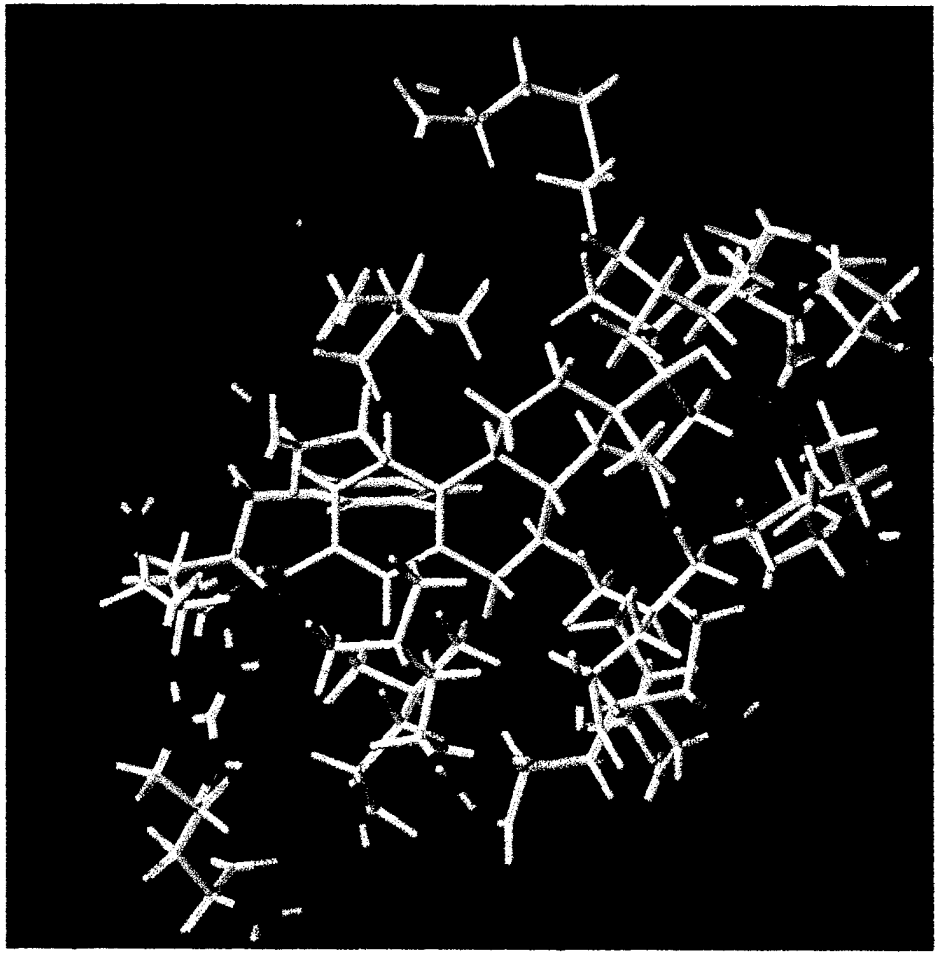
E. Grants:

Agency	**US Army Medical Research & Materiel Command**
Grant No.	DMAD17-99-1-9342, Breast Cancer Program
Title	**Predoctoral Training Grant** for Ms. Jennifer L. Windholtz: Antiestrogenic isoflavinoid analogs: Targeted drug design for inhibition of human breast cancer cell proliferation
Date	July 1, 1999 to June 30, 2002
Amount	$65,989 total costs for 3 years
Role	PI, Mentor

Agency	**US Army Medical Research & Materiel Command**
Grant No.	DMAD17-00-1-0388
Title	Novel Therapeutic Agents for Breast Cancer from Benzopyrone Combinatorial Libraries
Date	May 1, 2000 to April 30, 2003
Amount	$74,889 (annual direct costs)
Role	Principal Investigator

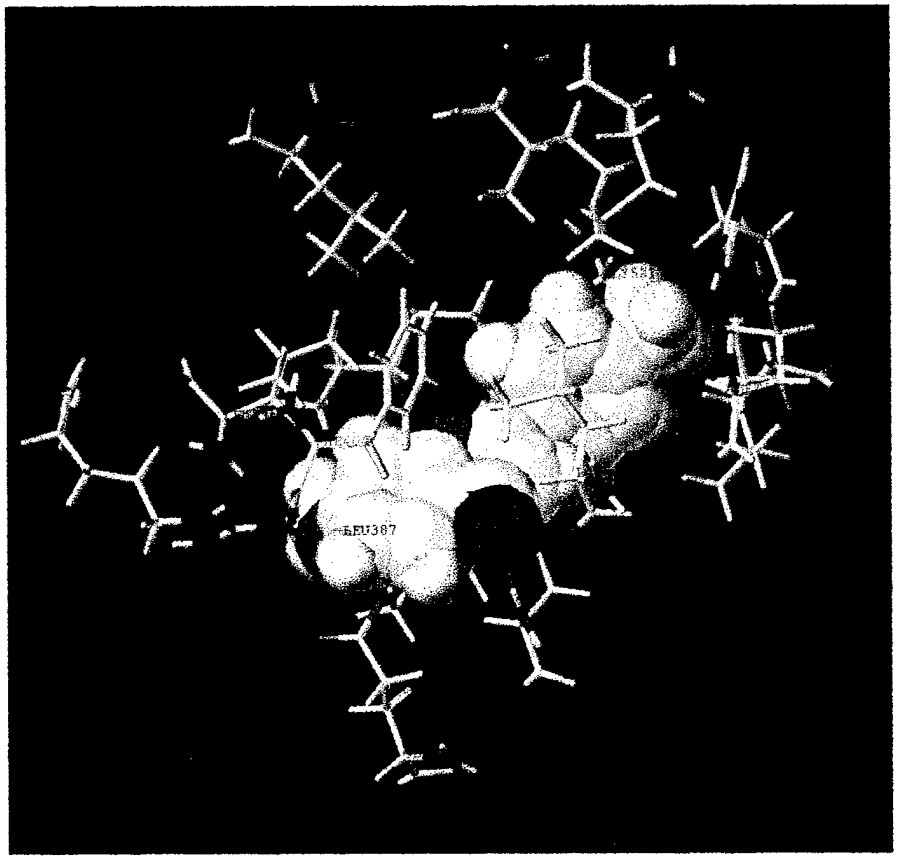
E_2 in binding site of ERα LBD

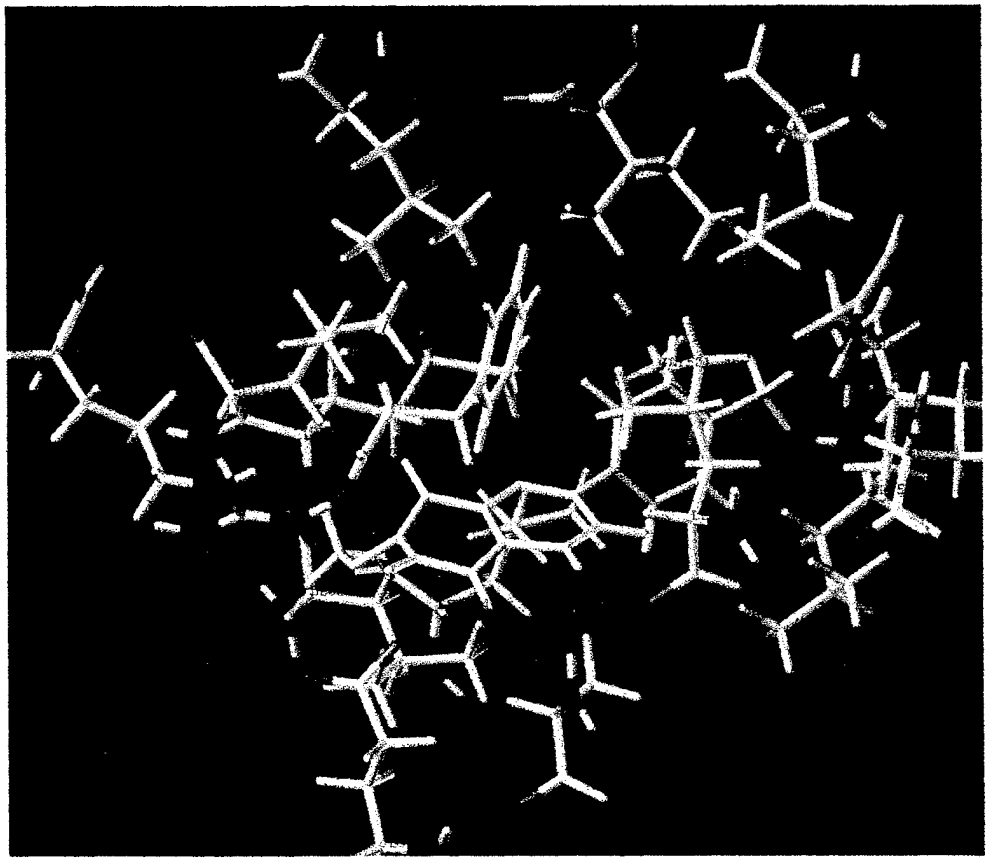




Benzopyrone in binding site of ERα LBD

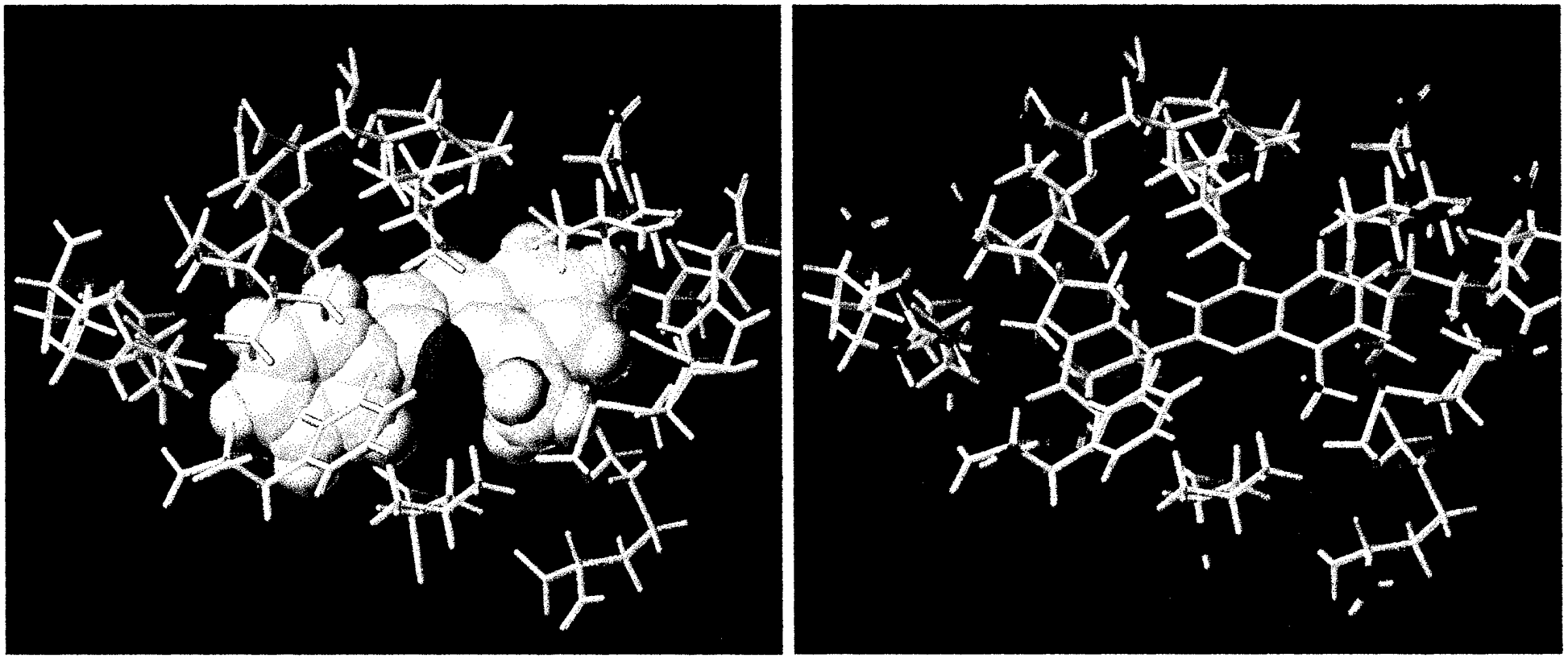
Orientation *A*



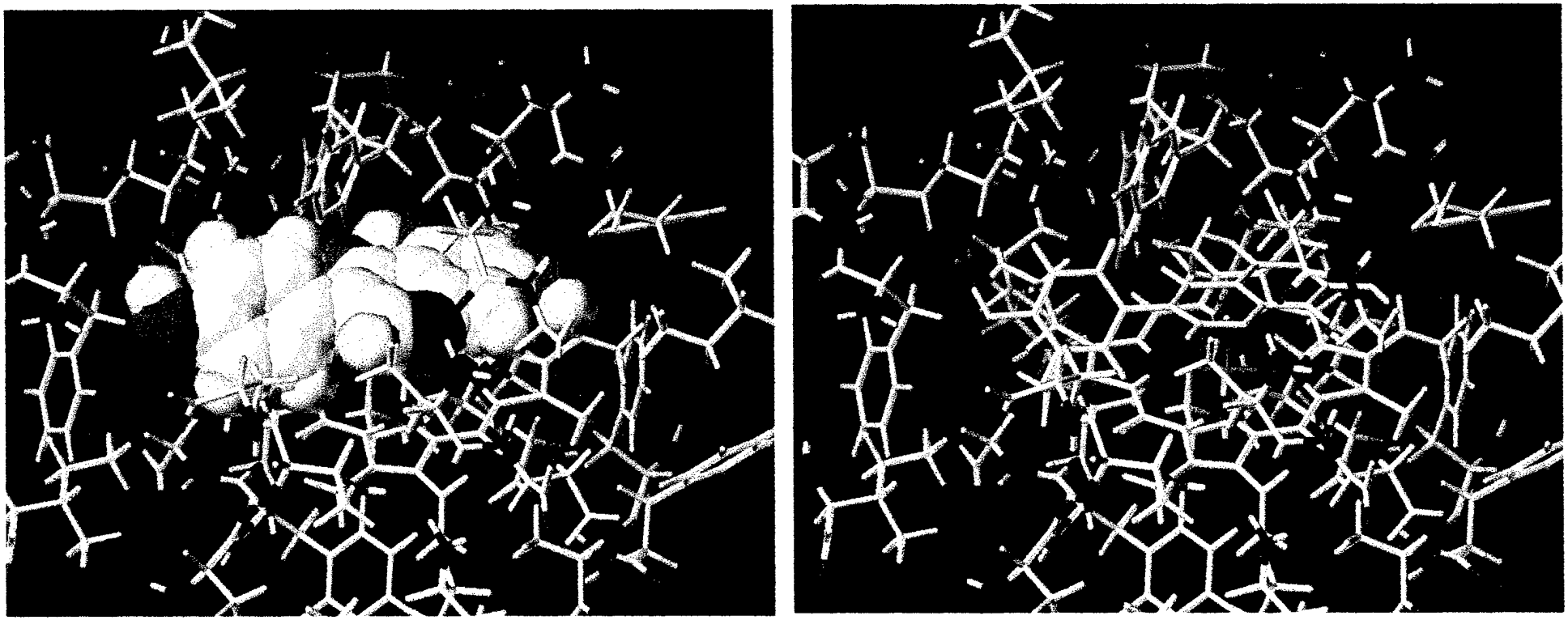


Benzopyrone in binding site of ERα LBD

Orientation *B*



Benzopyrone in binding site of Aromatase



COPIES OF MANUSCRIPTS AND ABSTRACTS OF PRESENTATIONS

Copies of Manuscripts:

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J.L. Whetstone, A.S. Bhat, and **R.W. Brueggemeier**, Benzopyrone as a scaffold for combinatorial libraries. 1999 National Organic Symposium, Madison, WI, June 13-17, 1999, Abst. #287.

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R.W. Brueggemeier, J.L. Whetsone, A.S. Bhat, and S. Joomprabutra, Benzopyrone combinatorial libraries for identification of selective agents for molecular targets in breast cancer. Molecular Targets in Cancer Therapeutics, AACR-NCI-EORTC International Conference, Washington, DC, November 16-19, 1999, Abst. #321.

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Pergamon

Tetrahedron Letters 40 (1999) 2469–2472

TETRAHEDRON LETTERS

Novel Synthetic Routes Suitable for Constructing Benzopyrone Combinatorial Libraries

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Received 14 December 1998; revised 22 January 1999; accepted 27 January 1999

Abstract

A series of O-(*t*-butylsilyloxy)benzoyl chlorides generated from the corresponding silyl esters were coupled with a range of terminal alkynes to afford the corresponding alkynyl ketones. The alkynyl ketones were converted to enaminoketones and then cyclized to yield the desired benzopyrone ring system. This synthetic protocol utilizes readily available starting materials, mild and high yielding reactions with good functional group tolerance, and is ideal for developing combinatorial libraries centered around the benzopyrone ring system.

Keywords: Benzopyrones; Flavonoids; Combinatorial chemistry; Sonogashira coupling

Synthesis and biological screening of a heterocyclic, small molecule library forms the backbone of most combinatorial chemistry programs today [1,2,3]. Molecular scaffolds that have been shown to interact with different receptor systems whose natural ligands bear no resemblance with each other are termed as "privileged structures" [4]. There is substantial interest in synthesizing libraries of privileged structures, with the hope that screening of such libraries would yield ligands for a diverse collection of pharmacological targets. The driving force behind synthesis and screening of privileged structure libraries is the underlying promise of reducing the synthetic effort required to generate lead structures for a range of biological targets. Benzodiazepines are examples of privileged structures that have been explored using combinatorial methods [1,2,3].

The benzopyrone ring system represents a privileged structure that is yet to be fully exploited by combinatorial chemistry [5,6]. This ring system is present in a number of natural products

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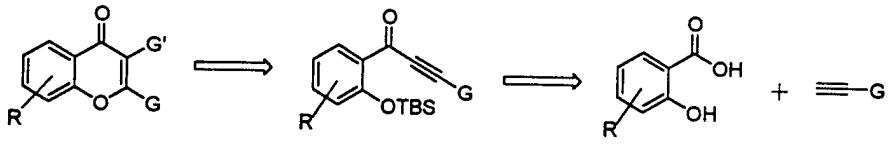
including flavonoids that interact with various enzymes and receptor systems of pharmacological significance.[2]

The benzopyrone ring system presents a fairly rigid molecular framework, resistant to hydrophobic collapse, with multiple sites to introduce potential diversity elements. The prevalent literature methods for constructing benzopyrones are not ideally suited for making libraries as these methods suffer from harsh reaction conditions, poor substituent tolerance and low yields [7].

Figure 1



Heteroannulation reactions of *o*-iodophenols and terminal alkynes in presence of CO are known to produce mixtures of 6-membered benzopyrones and 5-membered aurones [8,9]. In our synthetic planning we proposed to use salicyloyl chlorides as the coupling partner of terminal alkynes in order to obviate high CO pressure conditions required for heteroannulations (**Figure 1**). Also, the phenolic hydroxyl is masked as a TBS ether in order to prevent the oxypalladation reactions leading to mixtures of 5- and 6- membered ring systems. The desired benzopyrone would be then constructed by 6-endo-dig cyclization of the alkynone under controlled conditions that preclude the formation of aurones.

Salicylic acids were treated with 2.2 equiv of TBSCl and Et_3N in CH_2Cl_2 to generate the bisTBS protected salicylic acids ($\mathbf{A_1}$-$\mathbf{A_5}$) in quantitative yield [10]. The bisTBS salicylic acids were reacted with 1.2 equiv of oxalyl chloride in presence of catalytic amounts of DMF to provide the corresponding acid chlorides [11]. The acid chlorides were used for the Sonogashira couplings without any further purification (**Figure 2**). The acid chloride in Et_3N was reacted with a variety of terminal alkynes ($\mathbf{B_1}$-$\mathbf{B_7}$) in the presence of catalytic amount of $Pd(PPh_3)_2Cl_2$ and CuI [12]. It was important to use 3-5 mole excess of terminal alkynes and deoxygenate the reaction mixture in order to reduce the amount of alkyne homocoupled byproducts (Glaser Coupling). Salicylic acids ($\mathbf{A_2}$-$\mathbf{A_5}$) were coupled with phenyl acetylene ($\mathbf{B_1}$) to evaluate the effect of substitutions on the salicylic acid component over the coupling reactions. All of the coupling reactions gave desired alkynones in excellent yields (**Table 1, 2**). The acid sensitive NH-Boc function ($\mathbf{A_5}$) is successfully carried through the acid chlorination step, emphasizing the mild nature of reaction conditions. The one-pot acid chlorination-

[2] Molecules with benzopyrone ring system have shown to be active as tyrosine and protein kinase C inhibitors, antifungal and antiviral agents as well as antitubulin and antihypertensive agents. This nucleus can be exploited to develop novel antiinflammatory agents as well as selective modulators of estrogen receptors α/β, and adenosine receptor antagonists. Synthesis and screening of benzopyrone libraries would allow us to harvest the biological potential of these molecules.

Sonogashira coupling, key for introducing diversity, displays a wide substituent tolerance in both the coupling partners and provides the desired alkynones in excellent yields.

Figure 2

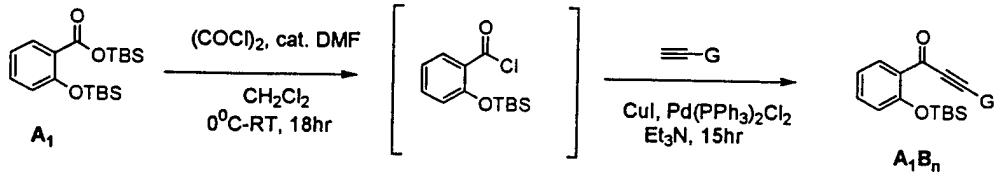


Table 1. Coupling of A_1 with terminal alkynes

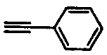
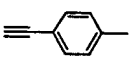
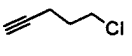
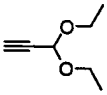
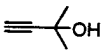
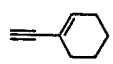
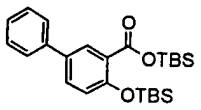
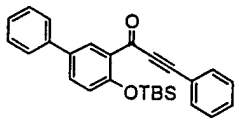
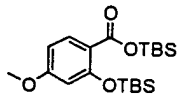
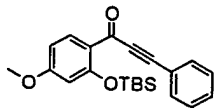
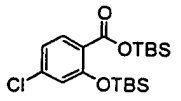
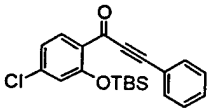
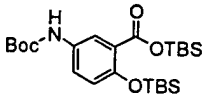
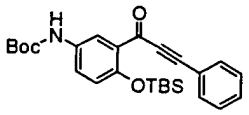
≡—G	Product A_1B_n (Yield)*
B_1 	A_1B_1 (92%)
B_2 	A_1B_2 (95%)
B_3 	A_1B_3 (85%)
B_4 	A_1B_4 (84%)
B_5 	A_1B_5 (78%)
B_6 	A_1B_6 (74%)
B_7 	A_1B_7 (96%)

Table 2. Coupling of B_1 with salicylic acids A_2-A_5

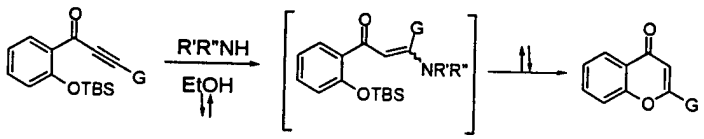
Salicylic acid	Product	A_nB_1 (Yield)*
A_2 		A_2B_1 (90%)
A_3 		A_3B_1 (92%)
A_4 		A_4B_1 (83%)
A_5 		A_5B_1 (78%)

* Isolated yields after workup and flash chromatography

Upon removal of TBS group, the free phenolic hydroxyl can effect 6-endo-dig or 5-exo-dig cyclization to yield either benzopyrones or aurones respectively [13]. We reasoned that, if the alkynones were first converted to enaminoketones and then subjected to TBS deprotection, the system would be prone to undergo Michael addition followed by elimination of secondary amine to exclusively yield the desired benzopyrones. To our surprise, we discovered that conversion of the alkynones to enaminoketones and subsequent cyclization could be effected in a single step. Thus, ethanolic solutions of alkynones refluxed with 10 equiv of diethylamine for 24 hours underwent cyclization to give the benzopyrones via enaminoketone intermediates. TLC revealed that the starting material was consumed within two hours and subsequent NMR analysis of the reaction mixture after 10 hours of reflux revealed a mixture of enaminoketone and benzopyrone. Pure samples of the enaminoketones were prepared by stirring an alcoholic solution of the alkynone with 10 equiv of the secondary amine for two hours. These

enaminoketones when refluxed with excess diethylamine formed benzopyrones. The results of these cyclizations are shown in **Figure 3**. Similar results were obtained by refluxing the alkynones with dimethylamine (2M solution in THF) and N-benzyl-ethylamine. This strategy effectively eliminates the 5-exo-dig cyclization option.

Figure 3. Cyclizations with Diethylamine



Alkynone
A_1B_n

Flavone

Alkynone	% Yield of Flavone*
A_1B_1	96%
A_1B_2	87%
A_1B_3	73%
A_1B_4	68%
A_1B_5	75%
A_1B_6	54%
A_1B_7	82%

*Isolated yield after workup and flash chromatography

In summary, we have disclosed a novel way to construct the benzopyrone nucleus. This method utilizes readily available starting materials, mild reaction conditions and displays a wide substituent tolerance and therefore should prove useful in constructing libraries of benzopyrones not readily accessible by conventional synthetic protocols.

Experimental: Oxalyl chloride (1.1mmol) was added dropwise to a cold (0^0C) solution of bisTBS salicylic acid (1mmol) in CH_2Cl_2 containing 3 drops of DMF. The resulting solution was stirred at 0^0C for two hours and stirred at room temperature for 16 hours. Solvent was evaporated, Et_3N(3mL) was added to the residue and argon was bubbled through the solution for five minutes. 5mmol of alkyne, 5mg $Pd(PPh_3)_2Cl_2$ and 5mg of CuI were added and the reaction mixture was deoxygenated by bubbling argon gas for 10min and stirred at room temperature for 12 h. MeOH (5mL) was added to the reaction mixture and solvents evaporated, the residue was taken up in diethylether, organics were washed with water, brine, dried (Na_2SO4) and concentrated, the residue was purified by flash chromatography (SiO_2; 18% EtOAc in Hexanes). All the coupling products were characterized by ^1H, ^{13}C NMR, IR and HRMS.

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A Method for the Rapid Synthesis of Benzopyrone Libraries Employing a Resin Capture Strategy

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Abstract

A novel resin capture strategy for synthesis of the benzopyrone ring system is described. The approach involves the use of an amine resin to capture alkynyl ketone intermediates during the synthetic procedure. Reaction of O-TBS-protected salicyloyl chlorides and terminal alkynes under Sonogashira conditions provides alkynyl ketones. The alkynyl ketones were treated with a piperazinyl resin to form support bound enaminones. Filtration and subsequent washings of the resin removed unreacted materials and impurities. The support bound enaminones upon heating in methanol underwent an on-resin cyclization to liberate the desired benzopyrones. Overall reaction yields of 60-70% were observed with substituted salicyloyl chlorides and substituted alkynes.

Introduction

The benzopyrone ring system is present in a number of natural products including flavonoids that interact with various enzymes and receptors of pharmacological significance. This ring system can serve as an important scaffold for developing novel small molecule therapeutic agents for the emerging molecular targets in breast and prostate cancer. We are interested in the construction and screening of combinatorial libraries focused on the benzopyrone ring system, with an aim to develop novel anticancer agents. Although there are numerous literature methods for synthesis of the benzopyrone ring system, they are not ideally suited for combinatorial approaches due to harsh reaction conditions, poor yields, and limited substituent tolerance.[1] In a recent communication we described a novel synthetic approach, suitable for constructing benzopyrone combinatorial libraries by solution phase chemistry.[2] We effected a one-pot conversion of bis-silylated salicylic acids into alkynyl ketones via an acid chlorination and subsequent Sonogashira coupling with terminal alkynes. The alkynyl ketones were treated with secondary amines to form enaminones, which underwent a facile cyclization and elimination of secondary amine to provide the benzopyrone nucleus (Figure 1). This method uses readily available starting materials, mild and high yielding reactions that display a high substituent tolerance, and is therefore ideally suited for rapid synthesis of diverse libraries. Since our initial report, two recent papers have described efforts in constructing benzopyrone libraries thus underscoring the importance of this scaffold in lead discovery.[3]

One method for adapting this chemistry onto solid phase involves the use of resin capture method, pioneered by Armstrong and co-workers as a tool in library synthesis.[4] In this approach, the synthesis is performed in the solution phase and the desired product from the reaction mixture is selectively transferred onto the solid phase. The product on the solid phase undergoes further chemical transformations and is subsequently cleaved off the solid support. This method can be viewed as a purification strategy wherein only the desired product from the reaction mixture is trapped onto a solid support, leaving the by-products and reactants in solution. Armstrong demonstrated the first successful use of resin capture method, wherein hexenamides formed in a solution phase Ugi reaction were trapped as esters onto a resin bearing an alcohol functionality. The trapped esters were released as free acids upon treatment with 20% TFA/DCM.[5] Armstrong's group also successfully synthesized a series of tetrasubstituted ethylenes employing resin capture.[6] Resin capture presents a practical alternative to both conventional solid phase and solution phase organic synthesis. Furthermore, the resin capture method was especially attractive for us as it would eliminate the need to adapt our solution phase benzopyrone chemistry onto solid-phase and yet be able to deliver the targeted libraries with minimal purification steps.

Our method for the synthesis of the benzopyrone ring system using secondary amines to effect cyclization of alkynyl ketones presented an opportunity to use a resin capture strategy. The proposed resin capture strategy is depicted in Scheme 1. Thus,

we envisioned secondary amines tethered to a solid support such as **A** can be expected to react with alkynyl ketones to form support bound enaminones of the type **B** (Scheme 1). The support bound enaminone could be easily separated from the excess reagents and by-products of the reaction mixture by simple filtration. On-resin cyclization of the enaminone would then release the benzopyrone and regenerate the secondary amine. The proposed resin capture method would facilitate a one-pot conversion of silylated salicylic acids to benzopyrones without requiring any intermediate purification steps. Such a method would be ideally suited for rapid, parallel, automated synthesis of benzopyrone libraries. This communication presents our preliminary results which serve as a proof of concept for the proposed resin capture strategy.

Results and Discussion

A piperazinyl Merrifield resin[7,8] was used as the support bound secondary amine in the studies to explore the feasibility of the proposed resin capture approach (Scheme 2). The solution phase synthesis of enaminones involved use of alcoholic solutions. In order to overcome the poor swelling properties of polystyrene resin in alcohols, a 1:1 solution of THF/methanol or THF/ethanol was used in the pilot reactions. A solution of the alkynone **2** in ethanol was canulated into a THF/ethanol solution of the resin **1**, and the resulting suspension was gently stirred under argon. In this particular experiment, the piperazinyl resin was present in 5-fold excess to the alkynone, mimicking the conditions used for solution phase synthesis. The progress of the

reaction was monitored by TLC (the alkynone should be trapped onto the solid phase and dissappear from the solution phase). The reaction was monitored for 24 hours; however, the alkynone remained unchanged as evidenced by TLC. Since methanol has better swelling properties for this resin as compared to ethanol, the same experiment was repeated by replacing ethanol with methanol.[9] At the end of 16 hours, a TLC analysis of the reaction revealed that the alkynone was completely consumed in the reaction. The resin sample was filtered, thoroughly washed with swelling and non-swelling solvents, and all the washings were pooled and concentrated. An NMR of the residue detected no starting alkynone, thus indicating that the alkynone had indeed reacted with the support bound amine and disappeared from the solution.

The resin sample was analyzed by IR (KBr pellet) and showed a strong absorption band at 1740 cm^{-1} that was absent in the parent piperazinyl resin **1**. This IR spectrum indicated the presence of a carbonyl containing compound bound to the solid support (intermediate **3**). Furthermore, IR analysis using a DRIFT accessory (diffuse reflectance accessory) enabled rapid analysis of small amounts of resin samples. DRIFT-IR, like the KBr pellet, detected the presence of a carbonyl frequency that was absent in the piperazinyl resin. The resin sample with the presumed enaminone was suspended in methanol and warmed to 40°C. The reaction was monitored by TLC, since the enaminone was expected to undergo cyclization and release the benzopyrone into the solution. A product spot on TLC with R_f value matching the benzopyrone **4** appeared after 45 minutes. The resin was heated for 16 hours, cooled and filtered. The

combined filtrates were concentrated and the residue was passed through a short pad of silica gel. Upon concentration, benzopyrone **4** was isolated in 82% total yield for both the resin capture and cyclative release steps. Post-cyclization DRIFT-IR analysis of the resin sample showed the carbonyl band (1740 cm^{-1}) had disappeared.

The scope of the resin capture strategy was further investigated based upon these initial results. Reaction conditions were modified such that the alkynone in solution is in excess compared to the support bound amine. A solution of alkynone **2** (0.7 mmol) in 1:1 THF/methanol was treated with 100 mg (0.07 mmol) of the resin **1** in its dry form. Three separate reactions were carried out and worked up after 2, 6 and 12 hours. The resin samples were collected by filtration, dried and analyzed by IR using the DRIFT accessory. In all the cases, the IR showed presence of carbonyl function on the support bound material. The resin samples were heated in methanol to afford cyclization, and each reaction provided the benzopyrone in 70-80% yield upon concentration of the filtrate (100% yield being 0.07 mmol). The fact that the two-hour reaction gives identical yields to the 12-hour reaction indicates that the enaminone formation is very rapid under these conditions. To further examine the scope of this reaction, piperazinyl resin **1** was reacted in separate reactions with a ten-fold excess of alkynones **5**, **6**, and **7** (Scheme 3). In all the cases, support-bound enaminones were detected by the presence of the carbonyl absorption band using IR, and subsequent cyclization provided the benzopyrones **8**, **9**, and **10** in yields of 60-70%.

These experiments demonstrate the utility of the resin capture strategy for the benzopyrone library synthesis. Different support bound amines, alternate solid supports, and other nucleophiles like support bound thiols can be examined to optimize the reaction conditions for the resin capture. This method has the potential to deliver a rapid, one-pot synthesis of benzopyrones from bis-TBS protected salicylic acids, eliminating the need for purification of intermediate alkynones. Furthermore, this method can be applied for synthesis of benzopyrones with no residual functionalities required for linkage to solid phase. The resin support is regenerated during the cyclization and can be recycled for additional rounds of resin capture. To the best of our knowledge, this is a first example of resin capture by secondary amines followed by elimination of the quaternized amine to generate a double bond. This method presents an interesting concept that can be exploited in a number of other synthetic applications in combinatorial chemistry.

Experimental Methods

General Information. The alkynone intermediates were prepared as previously described.[2] All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Lancaster Chemical Inc. (Windham, NH), analyzed for purity by TLC, and used as received unless otherwise indicated. The piperazinyl resin was received as a gift from Dr. Stephan Brase, Institut Organische Chemie, Aachen, Germany. Anhydrous solvents were dried by standard procedures. Alkyl amines were first stirred over phthalic anhydride, refluxed and distilled followed by distillation from CaH_2 and stored over KOH pellets. Silica gel TLC plates (60 F_{254}) were purchased from Analtech Inc (Newark, NJ) and visualized with a UV lamp and/or 5% ethanolic phosphomolybdic acid followed by charring. All intermediates were purified by flash column chromatography on silica gel (Merck Kieselgel 60) using the indicated solvent systems. Melting points were determined in open capillaries on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Laser Precision Analytical RFX-40 FTIR Spectrometer as indicated. ^1H NMR and ^{13}C NMR were recorded on an IBM AC/250 spectrometer at 250 and 67.5 MHz respectively in $CDCl_3$ solutions unless otherwise indicated using the residual protiosolvent signal as internal reference. Mass spectra were obtained at The Ohio State University Chemical Instrumentation Center on either a VG 70-2505, a Nicolet FTMS-200 or a Finnigan MAT-900 mass spectrometer.

2-Phenyl-4*H*-[1]-benzopyran-4-one (4):

Resin Capture: THF (3 mL) was added to 1 g of piperazinyl resin **1** (0.7 mmol/g), the resulting mixture was allowed to stand under argon with intermittent agitation for 45 min. A solution of alkynone **2** (50 mg, 0.14 mmol) in THF:EtOH (2:1, 1 mL) was cannulated into the resin sample. The reaction was maintained under argon, stirred with a magnetic stir bar, and followed for 24 h by TLC (hexane/EtOAc, 4:1). TLC indicated no reaction between alkynone and resin. The reaction mixture was then concentrated, resuspended in THF:MeOH (2:1, 3 mL), and allowed to stand under argon with intermittent agitation for 2 h. TLC (hexane/EtOAc, 4:1) of the reaction after 10 h detected a very small amount of the alkynone **2**. The reaction was stirred for additional 6 h. The resin was collected by filtration using a sintered glass funnel. The resin sample was thoroughly washed with alternating 10 mL portions of THF, CH_2Cl_2, EtOAc, and MeOH. The resin sample was dried (dry weight 950 mg). IR spectrum of this resin sample were compared with the piperazinyl resin (KBr pellet and DRIFT accessory) and showed a strong carbonyl absorption band at 1740 cm^{-1} indicating successful resin capture of the alkynone.

Cyclization: The resin sample from the above experiment was suspended in MeOH (10 mL) in a round bottom flask with a reflux condenser. The mixture was heated to 40°C for 10 h. After cooling, the resin was removed by filtration. The resin was thoroughly washed with swelling and non-swelling solvents. All the organics were combined, dried ($MgSO_4$) and concentrated. After a short flash column purification (SiO_2, hexane/EtOAc, 1:1) benzopyrone **4** was obtained as an off white solid (25 mg, 82%) mp 89°C; ^1H NMR ($CDCl_3$) δ 8.21 (dd, J = 1.4 Hz, 8.0 Hz, 1H), 7.90 (m, 2H), 7.69 (m, 1H), 7.63-7.46 (m, 5H), 6.79 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 178.4, 163.1, 156.3, 133.6, 125.7, 125.1,

124.0, 117.9, 107.6; IR (KBr, cm^{-1}) 3061, 1649, 1598, 1569, 1459, 1367, 1125, 780, 676; HRMS *m/z* (M+) calculated for $C_{15}H_{10}O_2$ was 222.0678, found 222.0678.

7-Methoxy-2-phenyl -4*H*-[1]-benzopyran-4-one (8):

Piperazinyl resin **1** (100 mg, 0.07 mmol) was added to solution of alkynone **5** (25 mg, 0.7 mmol) in THF:MeOH (1:1, 1 mL). The mixtures were stirred under argon for 12 h. The resin sample was recovered by careful filtration of the reaction mixture. The resin was washed thoroughly with swelling and non-swelling solvents, dried and examined by DRIFT IR. A carbonyl frequency at 1740 cm^{-1} was detected in the resin sample. The sample was suspended in MeOH (2 mL) in a round bottom flask fitted with a reflux condenser. The reaction was heated at 40°C for 10 h. The resin sample was removed by filtration and washed thoroughly. The combined organics were dried ($MgSO_4$) and concentrated. The residue was redissolved in CH_2Cl_2 and purified via flash chromatography (SiO_2, hexane/EtOAc, 1:1). Benzopyrone **8** was crystallized from ethanol to yield a white solid (13 mg, 76%): mp 102-104°C, ^1H NMR ($CDCl_3$) δ 8.05 (d, J = 9.0 Hz, 1H), 7.84 (m, 2H), 7.46-7.43 (m, 3H), 6.92-6.88 (m, 2H), 6.68 (s, 1H), 3.86 (s, 3H); ^{13}C NMR 177.7, 164.1, 162.9, 157.9, 131.8, 131.3, 128.9, 127.0, 126.1, 117.8, 114.3, 107.5, 100.4, 55.8; IR (KBr, cm^{-1}) 3460, 3058, 2959, 2928, 2866, 1726, 1651, 1602, 1564, 1444, 1372, 1354, 1273, 1162, 765, 666; HRMS *m/z* (M+) calculated for $C_{16}H_{12}O_3$ was 252.0783, found 252.0782.

2-(3-Chloropropyl)-4*H*-[1]-benzopyran-4-one (9):

Piperazinyl resin **1** (100 mg, 0.07 mmol) was added to solution of alkynone **6** (25 mg, 0.7 mmol) in THF:MeOH (1:1, 1 mL), and the reaction performed in the same manner as above. The filtrate was concentrated to provide benzopyrone **9** as a yellow oil (11.5 mg, 75%): ^{1}H NMR 8.12 (dd, J = 7.9, 1.7 Hz, 1H), 7.63-7.56 (m, 1H), 7.39-7.30 (m, 2H), 6.15 (s, 1H), 3.58 (t, J = 6.3 Hz, 2H), 2.78 (t, J = 7.8 Hz, 2H), 2.18 (q, J = 7.8, 6.2 Hz, 2H); ^{13}C NMR 178.8, 163.6, 156.4, 134.3, 125.9, 125.3, 124.1, 119.0, 110.8, 42.0, 31.3, 29.4; IR (KBr, cm^{-1}) 2940, 2858, 1730, 1663, 1610, 1465, 1379, 1128, 1078, 770; HRMS *m/z* calculated for $C_{12}H_{11}ClO_2$ was 222.0445, found 222.0442 (M+, 100%), calculated was 224.0415, found 224.0417 (M+2, 34%).

2-(1-Cyclohexenyl)-4*H*-[1]-benzopyran-4-one (10):

Piperazinyl resin **1** (100 mg, 0.07 mmol) was added to solution of alkynone **7** (25 mg, 0.7 mmol) in THF:MeOH (1 mL), and the reaction performed in the same manner as above. The filtrate was concentrated to provide benzopyrone **10** as a yellow oil (11 mg, 70%). ^{1}H NMR ($CDCl_3$) δ 8.12 (dd, J = 1.4 Hz, 8.0 Hz, 1H), 7.58 (m, 1H), 7.39-7.29 (m, 2H), 6.92 (m, 1H), 6.23 (s, 1H), 2.27-2.22 (m, 4H), 1.81-1.53 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 178.7, 163.6, 155.9, 133.8, 133.4, 129.6, 125.4, 124.5, 123.7, 117.1, 106.0, 25.9, 24.1, 22.1, 21.4; IR (KBr, cm^{-1}) 3436, 3058, 2928, 2859, 1726, 1664, 1564, 1471, 1397, 1366, 1224, 1131, 790, 753; HRMS *m/z* (M+) calculated for $C_{15}H_{14}O_2$ was 226.099, found 226.099.

Acknowledgements:

This research was supported by USAMRMC DAMD17-96-1-6136, USAMRMC DAMD17-98-1-8139, and NIH grant R21 CA66193. The authors would like to acknowledge Dr. Stephan Brase, Institut fur Organische Chemie der Technischen Hochschule, Aachen, Germany, for providing a sample of the piperazinyl resin and Dr. Robert Curley for providing the DRIFT accessory for analyzing resin samples.

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Figures and Schemes

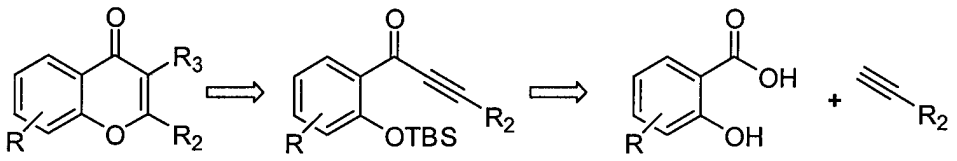
Figure 1: General scheme for benzopyrone synthesis.

Scheme 1: Proposed resin capture strategy for benzopyrone synthesis.

Scheme 2: Synthesis of benzopyrone **4** using resin capture method employing piperazinyl resin **1**.

Scheme 3: Synthesis of benzopyrones **8**, **9**, and **10** using resin capture method employing piperazinyl resin **1**.

Figure 1



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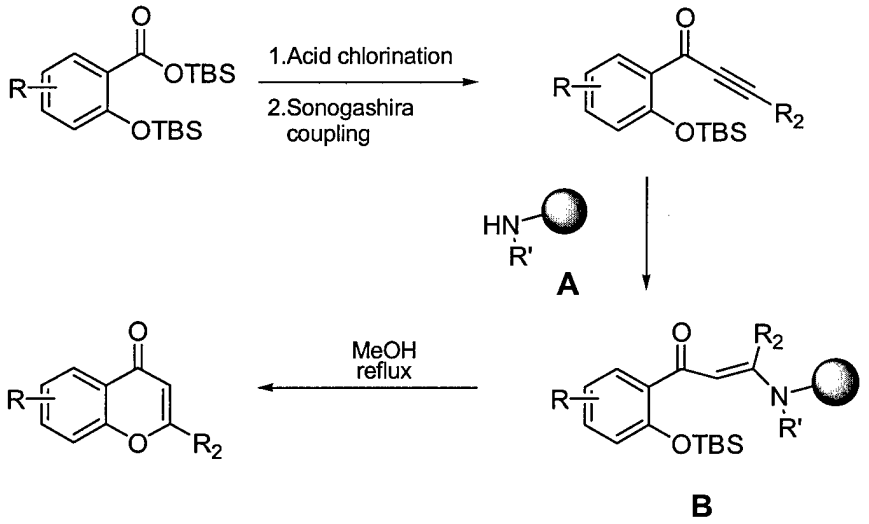
Scheme 1



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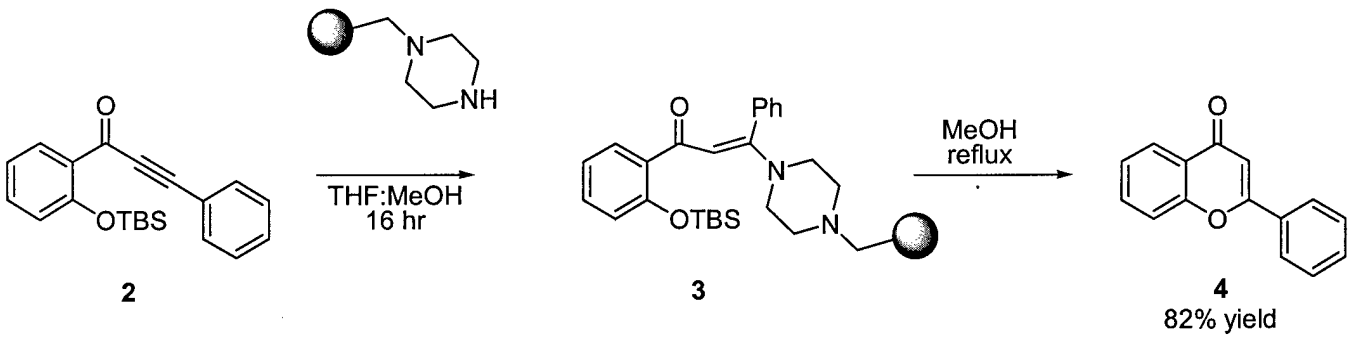

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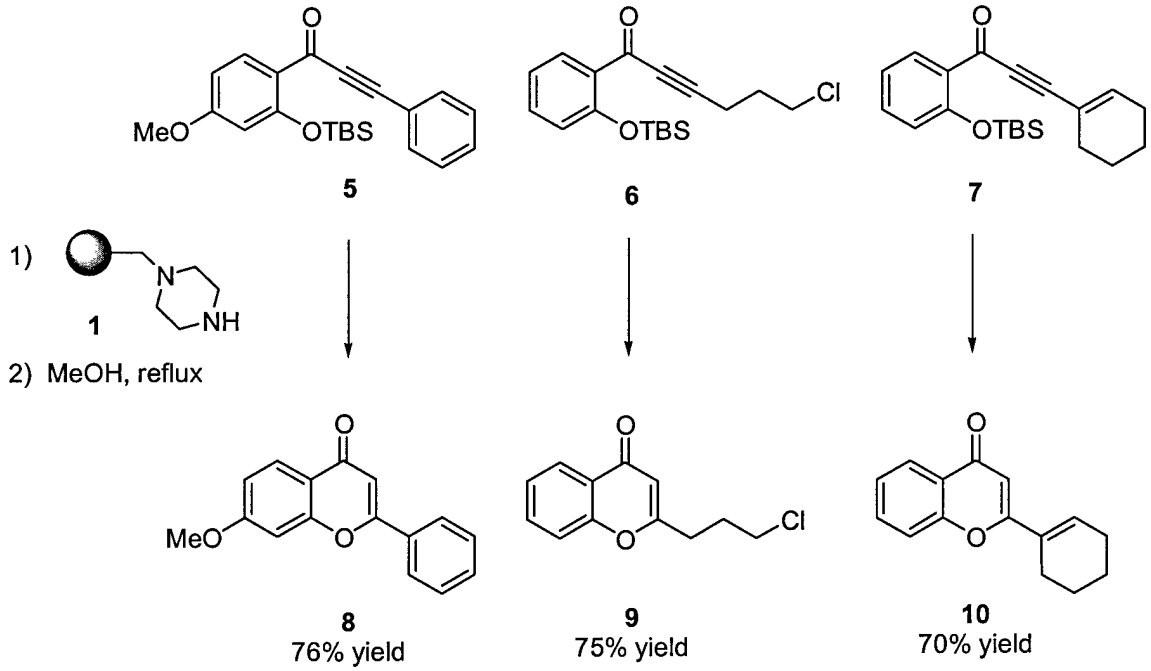
Scheme 3



A.S. Bhat, J.L. Windholtz, and **R.W. Brueggemeier**, Novel approaches for the synthesis of diverse benzopyrone libraries. 3rd Lake Tahoe Symposium on Molecular Diversity, Lake Tahoe, CA, Jan. 24-29, 1999.

A NOVEL APPROACHES FOR SYNTHESIS OF DIVERSE BENZOPYRONE LIBRARIES

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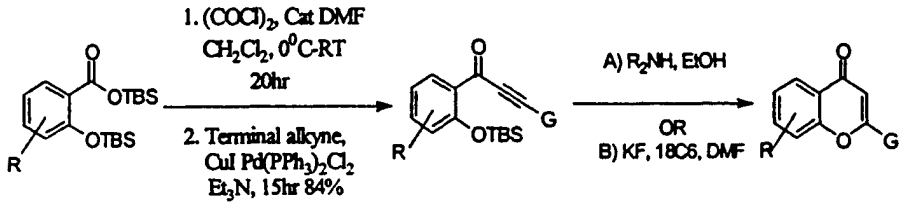
The benzopyrone ring system represents a privileged structure that has been under exploited by combinatorial chemistry. The current literature methods for constructing benzopyrones are not ideally suited for making adequately diverse libraries. A novel synthetic approach uses readily available salicylic acids and terminal alkynes as diversity inputs to construct benzopyrone nucleus. Silylesters of salicylic acids were converted to acid chlorides and coupled with a range of terminal alkynes to yield alkynyl ketones. Ethanolic solutions of alkynones refluxed with excess secondary amine cyclized to the desired benzopyrones. This synthetic approach utilizes readily available starting materials, mild reaction conditions and displays wide substituent tolerance on both the coupling partners and therefore should prove useful in constructing benzopyrone libraries not readily accessible by conventional synthetic methods.



J.L. Whetstone, A.S. Bhat, and **R.W. Brueggemeier**, Benzopyrone as a scaffold for combinatorial libraries. 1999 National Organic Symposium, Madison, WI, June 13-17, 1999, Abst. #287.

Benzopyrone as a Scaffold for Combinatorial Libraries.

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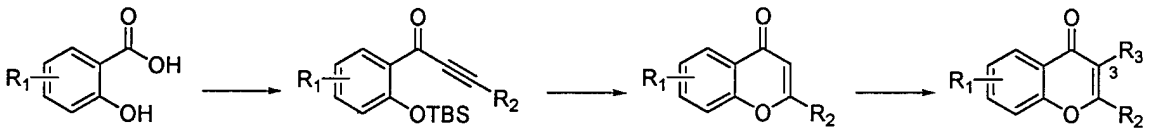
Synthesis and biological screening of a heterocyclic, small molecule library forms the backbone of most combinatorial chemistry programs today. The benzopyrone ring system represents a structural class that has been explored inadequately by combinatorial chemistry. This ring system is present in a number of natural products including flavonoids that interact with various enzymes and receptor systems of pharmacological significance. Synthesis and screening of substituted benzopyrone combinatorial libraries would allow us to harvest the biological potential of these molecules. The prevalent literature methods for constructing benzopyrones are not ideally suited for making libraries as these methods suffer from harsh reaction conditions, poor substituent tolerance, and low yields. Our novel synthetic route utilizes readily available salicylic acids and terminal alkynes as starting materials to construct the benzopyrone nucleus. Substituted salicylic acids are coupled with various terminal alkynes via a one-pot acid chlorination-Sonogashira coupling to give the desired alkynones in excellent yields. Conversion of the alkynones to enaminoketones and subsequent cyclization to the benzopyrone ring can be effected in a single step. This approach is characterized by mild and high yielding reactions with good functional group tolerance, and it is ideal for developing combinatorial libraries centered around the benzopyrone ring system. Addition of substituents such as hydroxyl and bromine at position 3 on the benzopyrone skeleton would be advantageous for introducing diversity into our library. Synthetic approaches for diversifying the benzopyrone skeleton and its application to construction of a flavonoid library will be presented. NIH R21 CA66193 and USAMRMC DAMD17-96-6136 supported this work.



R_3 = OH, Br

J.L. Whetstone, A.S. Bhat, and **R.W. Brueggemeier**, Synthetic routes for diversifying benzopyrones in combinatorial libraries. 218th American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999, Abst. MEDI #140.

Synthetic Routes for Diversifying Benzopyrone Combinatorial Libraries.

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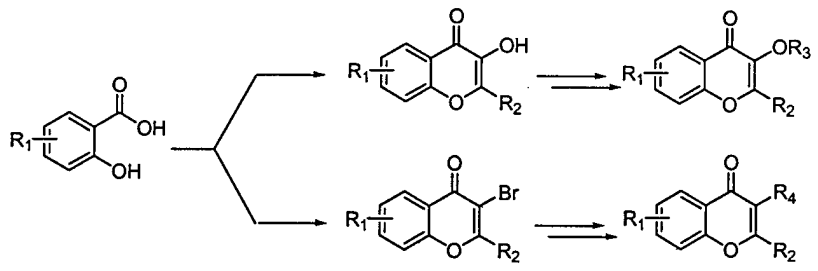
The benzopyrone ring system is an ideal molecular scaffold for the development of combinatorial libraries. This ring system is present in a wide range of natural products that interact with various enzymes and receptors of pharmacological significance. Synthesis and screening of substituted benzopyrone combinatorial libraries would be valuable in exploiting the biological potential of these molecules.

Synthesis of the benzopyrone skeleton involves a *6-endo-dig* cyclization of o-hydroxyphenyl alkynyl ketones under basic conditions. The precursor ketones are readily synthesized via one-pot Pd catalyzed Sonogashira couplings of substituted salicyloyl chlorides with various terminal alkynes. Substituents such as hydroxyl and bromine at position 3 on the benzopyrone skeleton will enable various elements of diversity to be introduced into our library. Synthetic routes for diversifying the benzopyrone skeleton and its application to constructing a flavonoid library will be presented. This work was supported by NIH R21 CA66193 and USAMRMC DAMD17-96-1-6136.



R.W. Brueggemeier, J.L. Whetsone, A.S. Bhat, and S. Joomprabutra, Benzopyrone combinatorial libraries for identification of selective agents for molecular targets in breast cancer. Molecular Targets in Cancer Therapeutics, AACR-NCI-EORTC International Conference, Washington, DC, November 16-19, 1999, Abst. #321.

Benzopyrone Combinatorial Libraries for Identification of Selective Agents for Molecular Targets in Breast Cancer.

Brueggemeier, R.W.; Whetsone, J.L.; Bhat, A.S.; Joomprabutra, S., College of Pharmacy, OSU Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210

Molecules with a benzopyrone ring system include a number of natural products such as flavonoids found widely in higher plants. The class of flavonoids encompasses the flavones, isoflavones, flavanones, and flavonols. Compounds in this class have shown activity as protein tyrosine kinase inhibitors, estrogen receptor agonists/antagonists, or inhibitors of steroidogenic enzymes. The low molecular weight, fairly rigid benzopyrone nucleus contains multiple sites of potential chemical diversity, and this chemical structure should serve as an ideal template for producing combinatorial libraries. Our hypothesis is that the design, synthesis, and screening of substituted benzopyrone combinatorial libraries would allow us to harvest the biological potential of these molecules and develop more selective agents for molecular targets in breast cancer. Our initial medicinal chemistry efforts developed synthetic methods for constructing benzopyrones that are accomplished under mild reaction conditions, allow for flexibility in substitutions, and provide for moderate to high yields. Our novel synthetic route utilizes readily available salicylic acids and terminal alkynes as starting materials to construct the benzopyrone nucleus. Substituted salicylic acids are coupled with various terminal alkynes via a one-pot acid chlorination-Sonogashira coupling to give the desired alkynones in excellent yields. Conversion of the alkynones to enaminoketones and subsequent cyclization to the benzopyrone ring can be effected in a single step. Synthetic approaches for diversifying the benzopyrone skeleton have been developed, and solution phase combinatorial chemistry is utilized to construct small flavonoid libraries. Bioassay determinations on synthetic benzopyrones include evaluation of effects on breast cancer cell proliferation, estrogen receptor agonist/antagonist properties, and inhibition of aromatase. This work was supported in part by NIH grants NCI R21 CA66193, USAMRMC DAMD17-96-1-6136, and USAMRMC DAMD17-99-1-9342.

R.W. Brueggemeier, Synthetic approaches to benzopyrone combinatorial libraries. Exploiting Molecular Diversity/High-Throughput Organic Synthesis, Cambridge Healthtech Institute, San Diego, CA, Feb. 10, 2000.

Synthetic Approaches to Benzopyrone Combinatorial Libraries for Identification of Selective Bioactive Agents

Robert W. Brueggemeier, College of Pharmacy, The Ohio State University, Columbus, OH

Abstract:

Molecules with a benzopyrone ring system include a number of natural products such as the bioactive flavonoids found widely in higher plants. Our initial medicinal chemistry efforts developed synthetic methods for constructing benzopyrones that are accomplished under mild reaction conditions allow for flexibility in substitutions and provide for moderate to high yields. Our novel synthetic route utilizes readily available salicylic acids and terminal alkynes as starting materials to construct the benzopyrone nucleus. Substituted salicylic acids are coupled with various terminal alkynes via a one-pot acid chlorination-Sonogashira coupling to give the desired alkynones in excellent yields. Conversion of the alkynones to enaminoketones and subsequent cyclization to the benzopyrone ring can be effected in a single step. Synthetic approaches for diversifying the benzopyrone skeleton have been developed, and solution-phase combinatorial chemistry is utilized to construct small flavonoid libraries.

R.W. Brueggemeier, Molecular modeling, steroid biochemistry, and drug design. 2000 DoD Era of Hope Breast Cancer Research Program Meeting, Atlanta, GA, June 8-12, 2000, Poster CC-26.

MOLECULAR MODELING, STEROID BIOCHEMISTRY, AND DRUG DESIGN

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This research examines the proteins involved in steroid hormone biochemistry and breast cancer at the structural and molecular level. An estimated 60-70% of human breast cancers are associated with sex hormone exposure. Approximately 60% of all breast cancer patients have hormone-dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth. Estradiol (E_2) is the most potent endogenous estrogen and is biosynthesized from androgens by the cytochrome P450 enzyme complex called aromatase. This enzyme is present in breast tissue and converts circulating androgens into estrogens. Estradiol produces normal physiological effects by binding to a specific nuclear receptor proteins, the estrogen receptors ERα and ERβ. Estrogens promote growth of estrogen-responsive (ER+) breast cancer cells, and production of growth factors by estrogens in established tumor cell culture lines has been demonstrated. The regulation of steroidogenic enzyme expression such as aromatase and on regulation of ER-mediated responses suggests critical roles for protein-protein interactions during gene expression. As these biochemical mechanisms become more fully understood, new macromolecules and biochemical pathways involving steroid-protein and protein-protein interactions will be identified and serve as new molecular targets for drug design and discovery.

The development of innovative therapeutic and/or chemopreventive approaches for breast cancer will involve the identification of novel macromolecular targets, the discovery of novel lead drug candidates, and drug design and development. This research project is providing the training, dedicated time, and computational resources for studies on steroid biochemistry, protein molecular modeling, and drug design and discovery. Current research endeavors are utilizing computational and experimental tools for macromolecular target identification, target validation, and lead identification. Initial efforts have focused on development of a molecular model of aromatase, identification of critical amino acids at the active site, examination of interactions with both steroidal and nonsteroidal inhibitors, and design of new analogs.

Support: The U.S. Army Medical and Materiel Command under DAMD 17-98-1-8139.

R.W. Brueggemeier, J.L. Whetstone, A.S. Bhat, and S. Joomprabutra, Combinatorial libraries for identification of nonsteriodal agents for breast cancer targets. 14th International Symposium of the *Journal of Steroid Biochemistry and Molecular Biology*, Quebec City, Canada, June 24-27, 2000.

COMBINATORIAL LIBRARIES FOR IDENTIFICATION OF NONSTERIODAL AGENTS FOR BREAST CANCER TARGETS. R.W. Brueggemeier*, J.L. Whetstone, A.S. Bhat, and S. Joomprabutra, Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA.

Molecules with a benzopyrone ring system include a number of natural products such as the bioactive flavonoids and isoflavonoids. Benzopyrone derivatives have shown activity as protein tyrosine kinase inhibitors, estrogen receptor agonists or antagonists, or inhibitors of steroidogenic enzymes. Our hypothesis is that the design, synthesis, and screening of substituted benzopyrone combinatorial libraries would allow us to harvest the biological potential of these molecules and develop more selective medicinal agents for molecular targets in breast cancer. Our initial medicinal chemistry efforts developed synthetic methods for constructing benzopyrones that are accomplished under mild reaction conditions, allowing for flexibility in substitutions. Our novel synthetic route utilizes readily available salicylic acids and terminal alkynes as starting materials, coupled via a one-pot acid chlorination-Sonogashira reaction to give the desired alkynones in excellent yields. Conversion of the alkynones to enaminoketones and subsequent cyclization to the benzopyrone ring is accomplished in a single step. Synthetic approaches for diversifying the benzopyrone skeleton have been developed, and solution-phase combinatorial chemistry is utilized to construct small flavonoid libraries. Bioassay determinations on synthetic benzopyrones include evaluation of effects on breast cancer cell proliferation, estrogen receptor affinity, and inhibition of aromatase. Several agents have shown selective effects on ER(+) breast cancer cell proliferation. This work is supported by grants DMAD17-99-1-9342 and DMAD17-00-1-0388 from the US Army Breast Cancer Program.